Technische Universiteit
Eindhoven
University of Technology

iGEM TU/e 2015
Biomedical Engineering

Eindhoven University of Technology
Room: Ceres 0.04
Den Dolech 2, 5612 AZ Eindhoven
The Netherlands
Tel. no. +31 50 247 55 59
2015.igem.org/Team:TU_Eindhoven

PCR Amplification

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1 PCR Amplification

Estimated bench time: 30 minutes
Estimated total time: 2 hours
Purpose: Amplification of DNA with the possibility of expanding the DNA sequence at the beginning and/or end with the primers.

It is essential to work with gloves at all times to protect the DNA from DNase activity.

1.1 Materials

- Autoclaved H_2O
- Autoclaved PCR tubes
- Bucket with ice
- DNA to be amplified
- Forward primer
- Pipettes and tips
- Q5 High-Fidelity 2X Master Mix
- Reverse primer
- Thermal cycler

1.2 Setup & Protocol

- Construct a PCR mixture in the following way. Start with the component with the largest volume and end with the Master Mix. Keep the Master Mix on ice.

Component	Quantity/mass/final concentration	Volume (µl)
H_2O	Fill up to 50 µl	
DNA	10 ng	
Primer FW	0.5 µM (10 µM stock)	2.5
Primer RV	0.5 µM (10 µM stock)	2.5
Q5 High-Fidelity 2X Master Mix	1X	25
Total		50

- Mix well by pipetting up and down.
- Run the following PCR program:

Step	Temp (°C)	Time (sec)	Cycles
Initial denaturation	98	120 (2 min)	1
Denaturation Annealing Extension	98 X[1] 72	10 15 20 sec/kb	35
Final extension	72	600 (10 min)	1
Cooling	4	hold	1

[1] The annealing temperature can be calculated for the set of primers using New England Biolabs Tm calculator. An annealing temperature of 3°C lower than the lowest melting temperature was used to increase yields.